

WHAT IS CLAIMED IS:

5 1. A process for folding chemically synthesized polypeptides, comprising treating a polypeptide and/or protein that comprises two or more derivatized cysteine residues with a reducing agent in a folding buffer having a predetermined pH and temperature.

10 2. The process as claimed in claim 1, wherein the derivatized cysteine residue corresponds to S-butyl-thio-cysteine residue.

10 3. The process as claimed in claim 1, wherein the reducing agent is cysteine.

15 4. The process as claimed in claim 1, wherein the folding buffer comprises one or more chaotropic salts.

15 5. The process as claimed in claim 4, wherein the chaotropic salts are chosen from the group consisting of guanidinium chloride and urea.

20 6. The process as claimed in claim 4, wherein the chaotropic salts in the folding buffer are present in a concentration of 0.1-1 M.

25 7. The process as claimed in claim 1, wherein the folding buffer has an alkaline pH.

25 8. The process as claimed in claim 7, wherein the pH lies between 7 and 9.

30 9. The process as claimed in claim 7, wherein the pH lies between 7 and 8.5.

10. The process as claimed in claim 1, wherein the temperature of the folding buffer lies between 25° and 40°C.

11. The process as claimed in claim 10, wherein the temperature lies
5 between 27° and 38°C.

12. The process as claimed in claim 10, wherein the temperature is about 37 °C.

10 13. A process for the preparation of biologically active proteins, comprising

(a) chemically synthesizing a polypeptide that comprises two or more derivatized cysteine residues;

(b) treating said polypeptide with a reducing agent in a folding buffer having a predetermined pH and temperature; and
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(c) purifying the obtained folded polypeptides and/or proteins.

14. The process as claimed in claim 13, wherein the derivatized cysteine residue corresponds to a S-butyl-thio-cysteine residue.
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15. The process as claimed in claim 13, wherein the reducing agent is cysteine.

16. The process as claimed in claim 13, wherein the folding buffer comprises one or more chaotropic salts.
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17. The process as claimed in claim 16, wherein the chaotropic salts are chosen from the group consisting of guanidium chloride and urea.

18. The process as claimed in claim 15, wherein
30 the chaotropic salts in the folding buffer are present in a concentration of 0.1-1 M.

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19. The process as claimed in claim 13, wherein the folding buffer has an alkaline pH.

5 20. The process as claimed in claim 19, wherein the pH of the folding buffer lies between 7 and 9.

21. The process as claimed in claim 20, wherein the pH lies between 7 and 8.5.

10 22. The process as claimed in claim 13, wherein the temperature of the folding buffer lies between 25° and 40°C.

15 23. The process as claimed in claim 22, wherein the temperature lies between 27° and 38°C.

24. The process as claimed in claim 22, wherein the temperature is about 37°C.

20 25. The process as claimed in claim 13, comprising the steps
(a) assembling S-t-butyl-thio cysteine polypeptide on an insoluble polymeric support by stepwise chain elongation;
(b) cleaving said S-t-butyl-thio cysteine polypeptide chain from said support by acidolysis;
(c) purifying the obtained S-t-butyl-thio cysteine polypeptide;
25 (d) folding the purified S-t-butyl-thio cysteine polypeptide by treating said polypeptide derivatives with a molar excess of cysteine in a folding buffer comprising a chaotropic salt and having an alkaline pH and a temperature of about 37° C; and
(e) purifying the obtained folded proteins by reverse phase High Performance Liquid Chromatography.

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27. The process as claimed in claim 25, wherein said polymeric support is a polyamide or polystyrene-based resin functionalized with the acid labile hydroxymethylphenoxyacetic acid linker.